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WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLUTION PREVENTION

MEMORANDUM

Date: May 6, 2011

SUBJECT: Amisulbrom: Cursory review of seven special studies designed to investigate the possible mode of action on the female reproductive tract and a special study to assess for behavioral reactions in the rat and several parameters for assessment of heart function in the dog.

PC Code: 016330
Decision No.: 423778
Petition No.: 9E7650
Risk Assessment Type: N/A
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MRID No.: See tables of MRID's on pages 2 and 3.

DP Barcode: 388343
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FROM: John Doherty 5/6/11
Risk Assessment Branch V
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THROUGH: Jack Arthur
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I. ACTION REQUESTED and CONCLUSIONS

The guideline toxicity studies to support the import tolerances for amisulbrom on grape and tomato were reviewed separately under TXR # 0055321 and DP Barcode 374160. The guideline multi generation reproductions study (2005, MRID 47918032) demonstrated that the high dose (15,000 ppm) F1 females had reproductive failure resulting in producing only two F2 litters. There were also indications of altered conditions and weight in the ovaries and uterus in the 3000 ppm dose group. The registrant also submitted seven studies (2005 or 2006, MRID Nos

479180-52 to -58) that were designed to address a possible mechanism for the adverse effects of amisulbrom on the female reproductive tract. The registrant did not request a mode of action analysis based on these studies. Therefore, these non-guideline studies were perused for their possible usefulness in the event that a mode of action analysis is requested in the future. The studies are listed in the table below. Comments on each study are presented in Appendix 1 (Summary of Studies Based on Cursory Review).

In addition, another non-guideline study (2005, MRID No.: 47918047) was submitted that assessed for behavioral reactions in male rats and several parameters on heart function in male dogs. The assessments in dogs included: heart rate, blood pressure, respiration rate and ECG. No DER was prepared from this study and no classification was assigned. The author maintains that neither the rat nor the dog was affected by amisulbrom treatment with regard to the parameters investigated. RABV reviewer notes, however, that there was a statistically significant increase in systolic and mean blood pressure at the highest dose (2000 mg/kg). Since the dose is very high, the toxicological significance is not apparent.

MRID Summary for special studies with amisulbrom

Study Type	MRID (year) Doses	Comments
Special Study: Effect on rat fetus ovary	47918052 (2005) Ovaries from study MRID # 47918051 where rats were dosed with 1500 mg/kg/day.	No marked changes in the mean number of primordial follicles and apoptosis.
Special Study: Confirmative effect on ovaries of new born rats - cross fostering.	47918053 (2005) 0 or 15,000 ppm gestation to postnatal day 21. Followed by cross fostering. 10 mg/kg busulphan as positive control .	Ovary weight decreased in amisulbrom treated group. Report asserts effect is due to body weight decrease not direct effect of amisulbrom. RABV considers data open to interpretation.
Special Study: Ovary development (dietary administration, cross fostering and dietary restriction) - rat	47918054 (2006) 0 or 15,000 ppm gestation day 0 to PND 21. Pups cross fostered and others on dietary restriction to parallel weight effects of 15,000 ppm.	The dietary restricted group was *reported* to demonstrate the same effects on body, ovary and uterus weight and histopathological alterations as the 15,000 ppm amisulbrom.
Special Study: Ovary development (gavage administration) - rat	47918055 (2006) 0 or 1500 mg/kg/day to dams through gestation to lactation day 20 and pups to PND 40 by gavage. Cross fostering included.	NOAEL > 1500 mg/kg/day. No alterations in ovary or uterus.
Special Study: Effect on genitals – 4 week dietary - rat	47918056 (2005) 0, 600 or 20,000 ppm for four weeks to 7 week olds.	In females, only effects were on body weight decrease and slight liver relative weight increase. No effects on ovaries. In males, increased testis and epididymis weights probably reflecting conservation with body weight decrease.
Special Study: Uterotropic antagonistic on female immature rats	47918057 (2005) 0, 60, 300 or 1500 mg/kg/day once daily for three days.	No effects on uterus hypertrophy. No control to assure that the positive control produced its expected result.
Special Study: Effect on uterus in	47918058 (2005)	No effects of amisulbrom on uterus

Study Type	MRID (year) Doses	Comments
immature rats.	0, 300 or 1500 mg/kg/day once daily for four days. Exemastane, an aromatase inhibitor was positive control .	weight. The positive control demonstrated a decrease in uterus wet weight but not dry weight. No untreated control.
Special Study: Non-Guideline Acute dosing in rats for behavioral assessment and in dogs or respiratory and heart rate, blood pressure and ECG parameters.	47918047 (2005) 0, 200, 600 or 2000 mg/kg to male rats and male dogs	Author's conclusion: No effects on behavior in rats. Note: Rat brain weights were not taken. Report claims no effects on respiratory rate, blood pressure, heart rate and ECG parameters in dogs. It is noted, however, that there was a statistically significant increase in systolic and mean blood pressure in the dogs dosed with 2000 mg/kg 180 minutes after treatment

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Appendix I
Summary of Studies Based on *Cursory* Review

The rat multi-generation reproduction study (2005, MRID No.: 47918032) demonstrated several effects on the female reproductive tract at the at dose levels of 3,000 and 15,000 ppm that included at 3000 ppm delayed vaginal opening and slight to moderate atrophy of the ovary in F1 progeny, altered weights of the uterus. At 15,000 ppm, the effects in the females were so severe that there was reproductive failure with only two litters produced.

In this series of seven special studies (2005 or 2006, MRIDs 47918052 to 47918058), that included both dietary or gavage dosing of rats (BrlHAnJcl(GALAS)) with amisulbrom (99.1% purity, Lot No.: 224T020619) during gestation and/or lactation and/or post weaning were conducted in attempts to further characterize the observations noted in the rat reproduction study (2005, MRID No.: 47918032) where it was demonstrated that there were effects on the weight of the ovary and uterus at 3000 ppm and at 15,000 ppm reproductive failure. A working hypothesis for some of these special studies was that the alterations seen in the ovary and uterus in the rat reproduction study were a secondary effect of the body weight loss due to amisulbrom and not a direct effect of amisulbrom on the ovary and uterus. Some of the studies included cross fostering.

RABV acknowledges these studies but declines to conclude that they explain all of the effects of amisulbrom on the female reproductive tract that were indicated in the rat multi-generation study. A full mode of action assessment that follows the IPCS format and its review by HED's mechanisms review committee would be required before a more definite conclusion can be made.

Comments on each of the seven studies based on a cursory review of the study reports are as follows (in MRID order):

(1) **Furukawa, S. The effect of NC-224 on the rat fetus ovary (Histopathological examination of fetus ovary in NC103TE-115). Biological Research laboratories, Nissen Chemical Industries, Japan, Study No.: NC105HP-227, June 1, 2005, MRID No.: 47918052. Unpublished.**

Description of the protocol. The ovaries from rat fetuses taken from a study identified as "Teratogenicity study of NC-224 in Han Wistar rats (NCI03Te-115)" from a group dosed with 1,500 mg/kg were examined after preparation in hematoxylin-eosin for the number of primordial follicles and apoptosis using an image analyzer. This study was submitted to EPA under MIRD 47918051 and the DER is in TXR # 0055321). The methods section indicated that "ovaries from two fetuses each were harvested from dams" and the identification numbers for 10 control and 1500 mg/kg/day dose groups were indicated.
Comments on the Results. Only nine fetuses in the control group and 11 in the group dosed with 1,500 mg/kg amisulbrom were examined. There were no marked changes in the mean number of primordial follicles and apoptosis bodies reported.

(2) Furukawa, S. Confirmative study of the effect of NC-224 on the ovaries of newborn rats. Biological Research laboratories, Nissen Chemical Industries, Japan, Study No.: NC105-229. October 21, 2005. MRID No.; 47918053. Unpublished.

Description of study protocol. In this study (2005, MRID No.: 47918053) two groups of pregnant rat (BrlHAn:WIST@Jcl(GALAS) dams were dosed as control or 15,000 ppm amisulbrom (99.1% purity, Lot No.: 224T020619) from day 0 of gestation to birth or to day 21 of lactation. A positive control group consisting of 10 mg/kg of busulphan (an anti-neoplastic drug) was also included. However, the rationale for using this particular drug as a positive control was not provided. Immediately after delivery the pups were set in specific groups for cross fostering. Four dams from the control group suckled pups from both the control group (C-1) and from dams that were dosed with amisulbrom (C-2). Control pups were also then suckled with dams that received the amisulbrom (T-1) and the last set consisted of pups that were from the amisulbrom dosed dams that remained with the amisulbrom dams (group T-2). Dams were assessed for clinical signs, body weight and gain and food consumption and at necropsy the mammary gland was weighted. The pups were examined for body weight and following necropsy at PND day 21 their ovaries were weighted and examined histologically. Liver weight assessments were also made.

Results in dams: Body weight was decreased during gestation (-12%) and tended to be decreased during lactation (-9.6% in T/T group) as was food consumption during gestation. During gestation, the dams were reported to receive a mean intake of 913.5 mg/kg/day and during lactation the intake was 1418.5 mg/kg/day of amisulbrom. There were no clinical signs reported. The study report asserted that there were no effects on mammary gland absolute weight or gross pathological alterations. However, inspection of Table 7 of the study report indicates that the groups designated as T-1 (-20%), T-2 (-9%) and the positive control (-16%) all had lower relative weight for the mammary gland. The significance of this is not known.

Results in pups at day 0 of lactation (prior to cross dosing). There was no effect of amisulbrom on the number of pups, their body weight or number of "primordial follicles of litters" at day 0 of lactation. The positive control (busulfan) nearly obliterated the number of primordial follicles (study report Table 9.)

Results in pups to PND 21. Table 1 (below) illustrates some of the findings in the 21 day old pups. The pups in the two groups that were continued on the amisulbrom exposure had marked weight *decreases* starting at PND 7 and the terminal PND 21 body weight is shown. Relative but not absolute liver weight was *increased.* Absolute ovary weight was *decreased* in the T-2 group by 25% and relative ovary weight appeared to be *increased* in both the T-1 (28%) and T-2 (21%) groups but the differences was not statistically significant. Histopathologically, the T-1 and T-2 groups dosed with amisulbrom during lactation were shown to have an *increase* in "No. follicles of litters (mm^2).

The positive control produced the expected positive results and it was in the opposite direction of the effects of amisulbrom.

Study report Interpretation: The interpretation of these findings by the study author was that "It was deduced that exposure to NC-224 (amisulbrom) during lactation induced decreases in absolute ovary weight of newborn pups due to body weight less, but NC-224 did not directly affect the ovaries of newborn pups".

RABV interpretation. The body weight loss in both the groups that were nursed by dams and being dosed with amisulbrom (i.e. C/T and T/T) had similar body weight decreases (i.e. both

about 36%) and similar effect on relative liver weight (i.e. 27% for C/T and 39% for T/T) but only the T/T group had a lower absolute ovary weight but both had non-statistically significant apparent increase in relative ovary weight. RABV does not concur with the study author's interpretation of these data. They do not seem to support an hypothesis that body weight rather than a direct effect of amisulbrom on the ovary is responsible for the weight of the ovary effect. Other effects of amisulbrom may still be factors. Since the effects of the positive control were not similar to amisulbrom, no analogy with this positive control was established in this study.

Table- Summary of effects on female pups on PND 21 following oral and lactation exposure in a cross fostering study.

Group	Body Wt. PND 21	Liver Wt Absolute Relative	Ovary Wt Absolute relative	Histopathology[b]
C/C[c] (C-1)	53.0±4.1	2.386±0.469 4.398±0.557	0.0187±0.0019 0.0351±0053	18.4±2.0 45.0±18.7 15.6±7.2 39.5±13.1
T/C (C-2)	50.8±3.8 ns	2.105±0.334(-11.8%)ns 4.122±0.381	0.0186±0.0019 0.0366±0.0023	19.9±2.7 50.2±6.1 7.2±2.6 (-54%) 42.6±7.1
C/T (T-1)	33.9±9.7*** (-36%)	1.917±0.447 (-19.7%) ns 5.570±0.763* (+27%)	0.0153±0.0036 (-18%) 0.0450±0.0106 (+28%)ns	25.2±1.3 (+37%)*** 44.2±8.4 12.7±8.5 43.1±15.0
T/T (T-2)	33.6±4.2*** (-37%)	2.060±0.314(-14.7%)ns 6.112±0.490*(+39%)	0.0149±0.0011*(-25%) 0.0424±0.0065 (+21%)ns	24.1±1.1 (+30.9%)** 48.4±10.3 9.2±4.3 42.4±6.5
PC[a]	41.1±6.6* (-22%)	Not determined	0.0029±0.0014 (-85%)*** 0.0070±0.003 (-80%)***	8.0±6.4 (-57%)* 14.0±3.8 (-69%) 11.3±16.0 74.7±12.2 (+89%)
Source	Table 10	Table 11 (absolute) Table 12 (relative)	Table 11 (absolute) Table 12 (relative)	Table 13

[a] Positive control – busulphan

[b] First line "No. follicles of litters (mm^2); second line – Primary follicles (%); third line – Secondary follicles (%); fourth line – aretic follicle (%)

[c] c C/C – dam and pups not treated; T/C - dam treated, pups not treated; C/T – dams not treated, pups treated; T/T – both dams and pups treated.

(3) **Furukawa, S. The effect of NC-224 on ovary development in juvenile rats (dietary administration). Biological Research laboratories, Nissen Chemical Industries, Japan, Study No.: NC105EX-337, May 31, 2006. MRID No.: 47918054. Unpublished.**

Description of study protocol: This non-guideline study (2006, MRID No.: 47918054) included both cross fostering and dietary restriction. In the cross fostering study, groups of 7 rats (BrlHAnJcl(GALAS) were mated and dosed at 15,000 ppm amisulbrom (in 0.5%

methylcellulose at a rate of 10 mL/kg, 99.1% purity, Lot No.: 224T020619) until weaning. The average intake of amisulbrom was 892.2 mg/kg/day during gestation and 2291.8 mg/kg/day during lactation. At weaning, one group (T/T) continued on the amisulbrom diet but the cross fostered group (T/C) continued on control diet without amisulbrom until PND 40. In the dietary study, none of the rats were dosed with amisulbrom and there were a total of six groups depending upon when their diets were restricted. These groups were:

C/C – no dietary restriction
C/R50 – 50% reduced diet postweaning
C/R33 – 33% " " "
R/C - reduced suckling during lactation/no postweaning restriction
R/R50 - " " " " /50% reduced diet postweaning
R/R33 - " " " " /33% " " "

During the lactation period "an excess number (this number was not specified) of suckling rats were assigned to one lactating rat for food restrictions." After weaning, food restriction was attained for the 50% group by allowing the pups to feed *ad libitum* I day every 2 days and for the 33% group two days every 3 days until PND 40.

Results in dams: Body weight was decreased during gestation (after day 6) for the amisulbrom treated group. During lactation, lower body weight was noted on days 5 and 12 and on day 21 in the food restriction group. Food consumption was decreased during pregnancy in the amisulbrom group during pregnancy and lactation. For the food restricted group, increased food consumption in the dams was noted on day 21 of lactation. No significant effect on milk yield was noted but a tendency toward lower yield was apparent.

Results in pups during the lactation period: No effects on *survival* were noted in the pups in the group dosed with amisulbrom. In the food restriction group, some of the pups died and the number of suckling pups per lactating rat ranged from 15 to 20 at weaning. *Body weight* was significantly decreased in the male rats on PND 5 and in female rats "on and after PND 0" for the amisulbrom treated rats. In the food restriction group, significantly decreased body weight gain was noted in both sexes after PND 5 and change in body weight was said to be similar in both the food restriction and dosed groups. Absolute *stomach weight* was significantly decreased with a tendency for relative weight also being decreased in the rats dosed with amisulbrom at PND 4 but not in the food restricted group. There were no marked changes in the histopathology of the ovary at PND 4 with respect to number of follicles per unit and the proportions of various follicles (primodial, primary, and apoptotic) in the rats dosed with amisulbrom. *Eyelid opening* was reported as being delayed in both food restricted and amisulbrom dosed groups being statistically significant in the amisulbrom group.

Results in developing rats PND 21-40: The rats in the R/R50 group survived only until PND 31. In the R/R33 group only two rats died and showed reduced motor activity and lower skin temperature. One rat in the C/R50 group died. There were no clinical signs in the other groups. Table 1 illustrates the several parameters affected in females by dietary restriction or treatment with amisulbrom in the postnatal growth period (PND 21-40).

Table 1 . PND 21-40 effects in female pups. From MRID # 47918054.

Group	Body W	Food Con	Vagina Opening	Ovary wt	Uterus Wt	Ovary Histopath.[d]
C/C	100%[c] 125.3	100%	0/6[a]	59.9±10.5[b] 47.97±8.89	0.31±0.13 0.25±0.11	All expressed as 100%.
CR50	58.9%	51.1%	3/6	23.7±6.5***	0.07±0.02**	Total: 225%***

	73.7***			32.54±10.31*	0.10±0.03*	PF: 230%??? SMF: 238%*** AF: 269%*** CL: 0%***
CR33	78.8% 98.7**	69.1%	0/6	35.6±9.8** 36.33±11.06	0.16±0.10* 0.16±0.11	Total: 180%* PF: 136%??? SMF: 185%** AF: 212%* CL: -47%**
T/C	86.8% 108.8*	86.6%	0/6	55.4±8.4ns 50.91±7.71	0.29±0.09ns 0.27±0.09	Total: -12%??? PF: -21%??? SMF: -13%??? AF: -16%??? CL: 8%???
T/T	79.9% 100.1**	73.2%	1/6	35.7±10.0** 35.27±8.57*	0.17±0.04* 0.17±0.03	Total: 157%* PF: -33%??? SMF: 186%??? AF: 174%* CL: -19%(ns)
R/C	84.7% 106.1*	87.4%	0/6	49.7±7.6 47.11±7.32	0.27±0.08ns 0.25±0.08	Total: 132%??? PF: -9%??? SMF: 135%??? AF: 140%??? CL: 114%???
R/R50	All pups die by day PND 31.					
RR33	60.4% 75.7***	58.1%	3/6	25.3±10.2*** 33.5±9.82*	0.11±0.05* 0.13±0.04	Total: 321%??? PF: 115%??? SMF: 300%??? AF: 421%??? CL: -93%***
Data Source	p.20 p.35	p.21	p.38	p.39 p.40	p.39 p.40	p.41

[a] Vaginal opening, number *not* opened by PND 40/number available.
[b] Total of left and right ovaries (mg). Top line-absolute weight; bottom line – relative weight
[c] Control expressed as 100% and percent differences is in top line. The mean absolute weight is in bottom line (variance in s not included but statistical notation suggests variance.
[d] Two tables of histpathology of the ovary were presented. One illustrating data in more absolute terms (mm^2) and the other in relative terms (% "follicle concentration ratio"). Data in the former table were for total number of ovary (Total), primary follicle, secondary mature follicle (SMF), atretic follicle (AF) and corpora lutena (CL). Only changes noted for each group are listed above and expressed as a percent of the control group (C/C).
*P < 0.05, *** P<0.001, ??? statistical notation was not included but difference is large.

The histopathology data indicate some values as showing statistical significance and others of nearly the same magnitude in difference from the control not showing statistical significance.

The key findings in Table 1 are that:

-Body weight and food consumption was decreased in all groups.

-The weight effect in the C/R50 group was accompanied by delayed vaginal opening, decreased ovary and uterus weight and more indications of histopathological changes. Lesser effects were evident in the in the C/R33 group.

-Stopping exposure to amisulbrom (T/C) group resulted in an apparent recovery of body weight, no effects on the ovary or uterus weights and histopathological alterations did not reach statistical significance *according to the study report*. The histopathological values reported (see

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table for the T/C group) were generally lower and in the opposite direction of the animals on diet restriction than the control but it is noted that there is a small magnitude of the differences.

-Maintaining the pups on amisulbrom exposure (T/T group) resulted in lower body, uterus and ovary weight and *some but not all* indications of histopathological alterations in the same direction but of lower magnitude than the dietary restriction groups (CR50 and CR33).

- The lactation restriction group (R/C) also showed lower body weight loss and lesser effects on the ovary, uterus weights and histological alterations did not reach statistical significance.

-Lactational and growth dietary restriction group (R/R33) demonstrated reduced body weight and ovary and uterus weights and there were increased in the same direction and close to the same magnitude in the histopathological findings of the ovary but statistical significance was not attained except for corpora lutena which were almost obliterated.

RABV Comments: This study is classified as Acceptable/Nonguideline. The study contains useful information comparing the animals dosed with amisulbrom with animals deprived of nutrition during lactation and/or post-weaning up to 40 days with respect to body, uterus and ovary weight and histopathology.

(4) **Furukawa, S. The effect of NC-224 on ovary development in juvenile rats (gavage administration). Biological Research laboratories, Nissan Chemical Industries, Japan, Study No.: NC105EX-377, May 31, 2006, MRID No.: 47918055. Unpublished.**

Description of study protocol. In this non-guideline study (2006, MRID No.: 47918055) that included *cross fostering*, groups of 7 rats (BrlHAnJcl(GALAS) were mated and dosed as control or 1,500 mg/kg/day amisulbrom (in 0.5% methylcellulose at a rate of 10 mL/kg, 99.1% purity, Lot No.: 224T020619) from days 0 of gestation to day 20 of lactation. The pups (6 females/group) were then dosed from days 21 to day 40. The cross fostering was achieved by continuing a group on as control/control (C/C), continuing the treatment at the same dose (T/T) and discontinuing treatment of the pups until day 40 (T/C).

Results. Numerous assessments of the dams were made but there were no marked effects on body weight or gain, food consumption (one day effect only), clinical signs, and delivery of pups or milk yield.

Assessment of the content of the stomach of the rat pups indicated that parent NC-224 and the metabolites IT-4 and IT-5 were present. The study report asserted that since NC-224 is metabolized "immediately" after absorption, the parent compound in the pup's stomach resulted from ingestion of the dam's feces.

The pups had only slight body weight effects but at PND 40 the T/C pups were 101.8% and the T/T pups but were slightly lower at 95.5% relative to the C/C group indicating a slight effect of daily dosing in the growing pup. During lactation (when all the pups were exposed), the males had a tendency toward decreased weight but statistical significance was not attained but the females had a statistically significant decrease in body weight gain. There was also no effect in the T/C group but a slight effect (~ -4%) in the T/T group in the growing pups on food consumption. There were no effects on developmental landmarks such as eyelid opening, vaginal opening, absolute or relative ovary or uterus weight or alterations noted in the histological examination of the ovary at PND 4 or 40. With the exception of slight changes in weight and food consumption there were no differences in the T/C and T/T group. **This study does not support a conclusion that amisulbrom at a dose level of 1,500 mg/kg/day during gestation,**

lactation and PND exposure to day 40 induced alterations in the ovary or uterus of rat pups.

(5) Usuda, K. Confirmative study of the effect of NC-224 on the genitals of rats (four-week administration in diet). Biological Research laboratories, Nissen Chemical Industries, Japan, Study No.: NC105SA-024, May 2, 2005, MRID No.: 47918056. Unpublished.

Description of study protocol. In the this study (2005, MRID No.: 47918056), three groups 8 male and female rats (BrlHAn:WIST@Jcl(GALAS) that were 7 weeks of age at the start of dosing were dosed as control, 600 or 20,000 ppm amisulbrom (99.1% purity, Lot No.: 224T020619) for four weeks. In-life investigations included assessment of body weight and gain, food consumption and clinical signs. Post necropsy included measurement of the major organ weights (liver, testes, epididymis, uterus (with and without lumen fluid) and ovary), histopathological assessment of the vagina and measurement of sex hormones (follicle stimulating hormone and luteinizing hormone (in both males and females) testosterone (in males) and progesterone and prolactin (in females).
Results - general. Decreases in body weight were noted in both males (-10.3% final weights) and females (-7.7% final weights) in the 20,000 ppm dose group only. Body weight gain over the four weeks was also reduced by 27% in males and 24% in females. Decreases in food consumption were also noted in this group. Sporadic decreases in food efficiency were noted including "immediately" following initiation of dosing and the body weight differences were noted by day 3. The mean achieved dosage of amisulbrom for males and females was 47.7 and 54.0 for the 600 ppm dose group and 1509.1 and 1757.0 for the 20,000 ppm dose group.
Results – male. Relative liver (13.7%***), testes (13.7%* based on total) and epididymis (16%* based on total) weights were significantly increased but not the absolute weight in the high dose. No Histopathological assessment of the testes or epididymis was made to establish pathological correlates with the weight effect. There were no alterations in the hormones.
Results – female. Only relative liver weight (18.9%) was increased at 20,000 ppm but not the absolute weight. No effects of treatment were evident by histological examination of vagina and there were no alterations in hormones.

(6) Usuda, K. Uterotrophic assay of NC-224 in female immature rats (antagonistic study). Biological Research laboratories, Nissen Chemical Industries, Japan, Study No.: NC105, April 25, 2005, MRID No.: 47918057. Unpublished.

Description of study protocol. In this study (2005, MRID No.: 47918057) groups of six 20 day old female rats (BrlHan:WIST@jcl(GALAS) were dosed as control, 60, 300 or 1500 mg/kg of amisulbrom (Lot No.: 224T020619, in 0.5% merhylcellulose once daily for three days. Thirty minutes after dosing with amisulbrom, a dose of ethinylestradiol was administered orally (in deionized water) to investigate whether amisulbrom inhibits the uterine hypertrophy activity of the estradiol compound. Two hours before necropsy, 5-bromo-2'deoxyuridine (BrdU) was administered in saline (ip). The animals were examined by necropsy and organs weighed. The uteri and vaginas were fixed in 10% neutral buffered formalin. HE staining and BrdU staining were done for the uterus and HE staining was done on the vagina. Proliferation activity of the

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uterine mucosal epithelium (RDS index) was assessed for at least 200 cells per animal and positive cells were scored using an image analyzer.
Results (systemic): Body weight *gain* was reduced (41% relative to the control) in the 1500 mg/kg dose group only. The low and mid dose groups both about 15% less than the control but there was no dose response between the two. The control group is considered high. There were no other effects of treatment including effects on uterus weight reported.
Results (utertrophic effects). There was no effect on uterus hypertrophy in the animals dosed with amisulbrom. The RDX index (proliferation activity of uterine mucosal epithelium) for the control was 5.1±1.6 and the high dose was 5.3±3.0.

RABV Comments: It is noted that there is no way to tell if anything is happening here with regard to the action of the ethinylestradiol inducing uterine hypertrophy since there was no control group that was not dosed with the steroid. In the absence of a demonstration that the uterine hypertrophy was actually induced, there is no way to tell if amisulbrom inhibits the uterine hypertrophy that was supposed to be induced and it can only be assumed.
(7) Usuda, K. Confirmative study of the effect of NC-224 on the uterus of female immature rats (aromatase). Biological Research laboratories, Nissen Chemical Industries, Japan, Study No.: NC105, April 22, 2005, MRID No.: 47918058. Unpublished.

Description of study protocol. In this study (2005, MRID No. 47918058) six rats (BrlHan:WIST@ jcl(GALAS) per group that were 27 days old at the initiation of dosing were dosed as control, 300 or 1500 mg/kg/day for once daily for four days. A positive control group consisted of rats dosed with exemastane, an inhibitor of aromatase. Two hours after dosing, androstenedione, a substrate for aromatase was administered subcutaneously and the antiaromatase activity assessed using uterus weights as indexes.
Results (systemic): There were no effects reported including on body weight gain.
Results: Both absolute and wet or dry uterus weights were not affected by amisulbrom treatment. The positive control group dosed with exemastane demonstrated reduced uterus *wet (including luminal fluid)* weight (both absolute – 27% and relative -28%). However, there was no effect on uterus dry weight for the positive control group.

RABV Comment: The positive control (exemastane) demonstrated an effect on uterus weight as an aromatase inhibitor. Amisulbrom did not show a similar effect as the aromatase inhibitor. Inclusion of assessment of aromatase activity in samples taken from the treated animals was not provided.

Additional Comments.

Note: All studies had statements of No Data Confidentiality. A GLP statement was included that stated that each study "does not meet the requirements of 40 CFR part 160". The statement provided that the studies were "performed in the spirit of GLP". Information on the qualifications of the staff of the laboratory was mentioned and that they are familiar with the principles of GLP for other types of studies. No Quality Assurance statements were provided.

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Chemical Name: Amisulbrom

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